



## Association between physical activity and risk of hepatobiliary cancers

### A multinational cohort study

Baumeister, Sebastian E.; Schlesinger, Sabrina; Aleksandrova, Krasimira; Jochem, Carmen; Jenab, Mazda; Gunter, Marc J.; Overvad, Kim; Tjønneland, Anne; Boutron-Ruault, Marie-Christine; Carbonnel, Franck; Fournier, Agnes; Kuehn, Tilman; Kaaks, Rudolf; Pischon, Tobias; Boeing, Heiner; Trichopoulou, Antonia; Bamia, Christina; La Vecchia, Carlo; Masala, Giovanna; Panico, Salvatore; Fasanelli, Francesca; Tumino, Rosario; Grioni, Sara; de Mesquita, Bas Bueno; Vermeulen, Roel; May, Anne M.; Borch, Kristin B.; Oyeyemi, Sunday O.; Ardanaz, Eva; Rodriguez-Barranco, Miguel; Chirlaque Lopez, Maria Dolores; Felez-Nobrega, Mireia; Sonestedt, Emily; Ohlsson, Bodil; Hemmingsson, Oskar; Werner, Marten; Perez-Cornago, Aurora; Ferrari, Pietro; Stepien, Magdalena; Freisling, Heinz; Tsilidis, Konstantinos K.; Ward, Heather; Riboli, Elio; Weiderpass, Elisabete; Leitzmann, Michael F.

*Published in:*

Journal of Hepatology

*DOI:*

[10.1016/j.jhep.2018.12.014](https://doi.org/10.1016/j.jhep.2018.12.014)

*Publication date:*

2019

*Document version*

Peer reviewed version

*Document license:*

[CC BY-NC-ND](#)

*Citation for published version (APA):*

Baumeister, S. E., Schlesinger, S., Aleksandrova, K., Jochem, C., Jenab, M., Gunter, M. J., Overvad, K., Tjønneland, A., Boutron-Ruault, M.-C., Carbonnel, F., Fournier, A., Kuehn, T., Kaaks, R., Pischon, T., Boeing, H., Trichopoulou, A., Bamia, C., La Vecchia, C., Masala, G., ... Leitzmann, M. F. (2019). Association between physical activity and risk of hepatobiliary cancers: A multinational cohort study. *Journal of Hepatology*, 70(5), 885-892. <https://doi.org/10.1016/j.jhep.2018.12.014>

## Association of Physical Activity and Risk of Hepatobiliary Cancers: A Multinational Cohort Study

Sebastian E. Baumeister<sup>1\*</sup>, Sabrina Schlesinger<sup>2\*</sup>, Krasimira Aleksandrova<sup>3\*</sup>, Carmen Jochem<sup>4</sup>, Mazda Jenab<sup>5</sup>, Marc J Gunter<sup>5</sup>, Kim Overvad<sup>6</sup>, Anne Tjønneland<sup>7</sup>, Marie-Christine Boutron-Ruault<sup>8</sup>, Franck Carbonnel<sup>9</sup>, Agnès Fournier<sup>8</sup>, Tilman Kühn<sup>10</sup>, Rudolf Kaaks<sup>10</sup>, Tobias Pischon<sup>11</sup>, Heiner Boeing<sup>3</sup>, Antonia Trichopoulou<sup>12</sup>, Christina Bamia<sup>12</sup>, Carlo La Vecchia<sup>13</sup>, Giovanna Masala<sup>14</sup>, Salvatore Panico<sup>15</sup>, Francesca Fasanelli<sup>16</sup>, Rosario Tumino<sup>17</sup>, Sara Grioni<sup>18</sup>, Bas Bueno de Mesquita<sup>19</sup>, Roel Vermeulen<sup>20</sup>, Anne M May<sup>21</sup>, Kristin B. Borch<sup>22</sup>, Sunday O. Oyeyemi<sup>22</sup>, Eva Ardanaz<sup>23</sup>, Miguel Rodríguez-Barranco<sup>24</sup>, María Dolores Chirlaque López<sup>25</sup>, Mireia Felez-Nobrega<sup>26</sup>, Emily Sonestedt<sup>27</sup>, Bodil Ohlsson<sup>28</sup>, Oskar Hemmingsson<sup>29</sup>, Mårten Werner<sup>30</sup>, Aurora Perez-Cornago<sup>31</sup>, Pietro Ferrari<sup>5</sup>, Magdalena Stepien<sup>5</sup>, Heinz Freisling<sup>5</sup>, Konstantinos K. Tsilidis<sup>31</sup>, Heather Ward<sup>32</sup>, Elio Riboli<sup>32</sup>, Elisabete Weiderpass<sup>33</sup>, Michael F. Leitzmann<sup>4</sup>

1. Chair of Epidemiology, Ludwig-Maximilians-Universität München, UNIKA-T Augsburg, Augsburg, Germany; Institute for Community Medicine, University Medicine Greifswald, Germany
2. Junior Research Group Systematic Reviews, Institute for Biometrics and Epidemiology, German Diabetes Center, Leibniz Center for Diabetes Research at Heinrich Heine University, Düsseldorf, Germany
3. German Institute of Human Nutrition Potsdam-Rehbruecke, Nuthetal, Germany
4. Department of Epidemiology and Preventive Medicine, University of Regensburg, Germany
5. Section of Nutrition and Metabolism, International Agency for Research on Cancer, Lyon, France
6. Department of Public Health, Section for Epidemiology, Aarhus University, Denmark
7. Diet, Genes and Environment, Danish Cancer Society Research Center, Copenhagen; Department of Public Health, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
8. CESP (Centre de Recherche en Epidémiologie et Santé des Populations), Université Paris-Sud, UVSQ, INSERM, Université Paris-Saclay, Villejuif, France; Institut Gustave Roussy, Villejuif, France
9. CESP (Centre de Recherche en Epidémiologie et Santé des Populations), Université Paris-Sud, UVSQ, INSERM, Université Paris-Saclay, Villejuif, France; Institut Gustave Roussy, Villejuif, France; Department of Gastroenterology, Bicêtre University Hospital, Assistance Publique des Hôpitaux de Paris, Le Kremlin Bicêtre, France
10. Division of Cancer Epidemiology, German Cancer Research Center, Heidelberg, Germany
11. Molecular Epidemiology Research Group, Max-Delbrueck-Center for Molecular Medicine in the Helmholtz Association (MDC), Berlin, Germany
12. Hellenic Health Foundation, Athens, Greece; WHO Collaborating Center for Nutrition and Health, Unit of Nutritional Epidemiology and Nutrition in Public Health, Dept. of Hygiene, Epidemiology and Medical Statistics, School of Medicine, National and Kapodistrian University of Athens, Greece
13. Hellenic Health Foundation, Athens, Greece; Dept. of Clinical Sciences and Community Health, Università degli Studi di Milano, Italy
14. Cancer Risk Factors and Life-Style Epidemiology Unit, Institute for cancer research, prevention and clinical network (ISPRO) Florence, Italy
15. Dipartimento di Medicina Clinica e Chirurgia, Federico II University, Naples, Italy
16. Cancer Epidemiology Unit, Department of Medical Sciences, University of Turin, Turin, Italy
17. Cancer Registry and Histopathology Department, "Civic - M.P. Arezzo" Hospital, ASP Ragusa, Italy
18. Epidemiology and Prevention Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Via Venezian, Milano-Italy
19. Centre for Nutrition, Prevention and Health Services, National Institute for Public Health and the Environment, Bilthoven, The Netherlands

20. Institute for Risk Assessment Sciences, Utrecht University, The Netherlands; Julius Centre for Public Health Sciences and Primary Care, University Medical Centre, Utrecht, The Netherlands
21. Julius Centre for Health Sciences and Primary Care, University Medical Centre Utrecht, Utrecht University, Utrecht, The Netherlands
22. Department of Community Medicine, Faculty of Health Sciences, University of Tromsø, The Arctic University of Norway, Tromsø, Norway
23. Navarra Public Health Institute, Pamplona, Spain; IdiSNA, Navarra Institute for Health Research, Pamplona, Spain; CIBER Epidemiology and Public Health CIBERESP, Madrid, Spain
24. Escuela Andaluza de Salud Pública, Instituto de Investigación Biosanitaria ibs, Granada, Hospitales Universitarios de Granada/Universidad de Granada, Granada, Spain; CIBER Epidemiology and Public Health CIBERESP, Madrid, Spain
25. Department of Epidemiology, Regional Health Council, IMIB-Arrixaca, Murcia University, Spain; CIBER in Epidemiology and Public Health (CIBERESP), Madrid, Spain
26. Unit of Nutrition and Cancer, Cancer Epidemiology Research Program, Catalan Institute of Oncology-IDIBELL, L'Hospitalet de Llobregat, Barcelona, Spain
27. Department of Clinical Sciences Malmö, Lund University, Lund, Sweden
28. Department of Internal Medicine, Skåne University Hospital, Lund University, Malmö, Sweden
29. Department of Surgical and Perioperative Sciences, Umeå University, Sweden
30. Department of Public Health and Clinical Medicine, Umeå University, Sweden
31. Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London, London, UK; Department of Hygiene and Epidemiology, University of Ioannina School of Medicine, Ioannina, Greece
32. Department of Epidemiology and Biostatistics, Imperial College London, London, UK
33. Department of Community Medicine, Faculty of Health Sciences, University of Tromsø, The Arctic University of Norway, Tromsø, Norway; Department of Research, Cancer Registry of Norway, Institute of Population-Based Cancer Research, Oslo, Norway; Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden; Genetic Epidemiology Group, Folkhälsan Research Center and Faculty of Medicine, University of Helsinki, Helsinki, Finland

\* Joint first authors

**Correspondence** to Dr. Sebastian E. Baumeister, Chair of Epidemiology, Ludwig-Maximilians-Universität München, UNIKA-T Augsburg, Neusässer Str. 47, 86156 Augsburg, Germany, Email: s.baumeister@unika-t.de

**Keywords:** Physical activity, hepatobiliary cancer, liver cancer, hepatocellular carcinoma

**Running head:** Physical activity and hepatobiliary cancer

**Word counts:** main text, references, tables/figure legends: 3,385; abstract: 197

**Number of figures:** 1, **number of tables:** 4

**Conflicts of interest:** All authors disclose no conflict. The sponsors had no role in the design of the study, the collection and analysis of the data, or the preparation of the manuscript.

**Financial support:** This work was in part supported by the French National Cancer Institute (L'Institut National du Cancer; INCA) (grant number 2009-139; PI: M. Jenab). The coordination of EPIC is financially supported by the European Commission (DG-SANCO) and the International Agency for Research on Cancer. The national cohorts are supported by Danish Cancer Society (Denmark); Ligue Contre le Cancer, Institut Gustave Roussy, Mutuelle Générale de l'Éducation Nationale, Institut National de la Santé et de la Recherche Médicale (INSERM) (France); Deutsche Krebshilfe, Deutsches Krebsforschungszentrum and Federal Ministry of Education and Research (Germany); the Hellenic Health Foundation (Greece); Associazione Italiana per la Ricerca sul Cancro-AIRC-Italy and National Research Council (Italy); Dutch Ministry of Public Health, Welfare and Sports (VWS), Netherlands Cancer Registry (NKR); LK Research Funds, Dutch Prevention Funds, Dutch ZON (Zorg Onderzoek Nederland); World Cancer Research Fund (WCRF); Statistics Netherlands (The Netherlands); Nordic Centre of Excellence programme on Food, Nutrition and Health, (Norway); Health Research Fund (FIS), PI13/00061

to Granada), Regional Governments of Andalucía, Asturias, Basque Country, Murcia (no. 6236) and Navarra, ISCIII RETIC (RD06/0020) (Spain); Swedish Cancer Society, Swedish Scientific Council and County Councils of Skåne and Västerbotten (Sweden); Cancer Research UK (14136 to EPIC-Norfolk; C570/A16491 and C8221/A19170 to EPIC-Oxford); Medical Research Council (1000143 to EPIC-Norfolk and MR/M012190/1 to EPIC-Oxford) (United Kingdom); Medical Research Council (1000143 to EPIC-Norfolk) (United Kingdom).

For information on how to submit an application for gaining access to EPIC data and/or bio-specimens, please follow the instructions at <http://epic.iarc.fr/access/index.php>.

**Author contributions:** Conception and design: Sebastian E. Baumeister, Sabrina Schlesinger, Krasimira Aleksandrova, Carmen Jochem, Mazda Jenab, Pietro Ferrari, Marc J Gunter, Magdalena Stepien, Heinz Freisling, Michael F. Leitzmann.

Analysis and interpretation of the data: Sebastian E. Baumeister, Sabrina Schlesinger, Krasimira Aleksandrova, Mazda Jenab, Pietro Ferrari, Marc J Gunter, Magdalena Stepien, Heinz Freisling, Michael F. Leitzmann.

Drafting of the manuscript: Sebastian E. Baumeister, Sabrina Schlesinger, Krasimira Aleksandrova, Michael F. Leitzmann.

Critical revision of the manuscript for important intellectual content: Carmen Jochem, Mazda Jenab, Marc J Gunter, Kim Overvad, Anne Tjønneland, Marie-Christine Boutron-Ruault, Franck Carbonnel, Agnès Fournier, Tilman Kühn, Rudolf Kaaks, Tobias Pischon, Heiner Boeing, Antonia Trichopoulou, Christina Bamia, Carlo La Vecchia, Giovanna Masala, Salvatore Panico, Francesca Fasanelli, Rosario Tumino, Sara Grioni, Bas Bueno de Mesquita, Roel Vermeulen, Anne May, Kristin B. Borch, Sunday O. Oyeyemi, Eva Ardanaz, Miguel Rodríguez-Barranco, María Dolores Chirlaque López, Mireia Felez-Nobrega, Emily Sonestedt, Bodil Ohlsson, Oskar Hemmingsson, Mårten Werner, Aurora Perez-Cornago, Pietro Ferrari, Magdalena Stepien, Heinz Freisling, Kostas Tsilidis, Heather Ward, Elio Riboli, Elisabete Weiderpass.

Final approval of the manuscript: Sebastian E. Baumeister, Sabrina Schlesinger, Krasimira Aleksandrova, Carmen Jochem, Mazda Jenab, Marc J Gunter, Kim Overvad, Anne Tjønneland, Marie-Christine Boutron-Ruault, Franck Carbonnel, Agnès Fournier, Tilman Kühn, Rudolf Kaaks, Tobias Pischon, Heiner Boeing, Antonia Trichopoulou, Christina Bamia, Carlo La Vecchia, Giovanna Masala, Salvatore Panico, Francesca Fasanelli, Rosario Tumino, Sara Grioni, Bas Bueno de Mesquita, Roel Vermeulen, Anne May, Kristin B. Borch, Sunday O. Oyeyemi, Eva Ardanaz, Miguel Rodríguez-Barranco, María Dolores Chirlaque López, Mireia Felez-Nobrega, Emily Sonestedt, Bodil Ohlsson, Oskar Hemmingsson, Mårten Werner, Aurora Perez-Cornago, Pietro Ferrari, Magdalena Stepien, Heinz Freisling, Kostas Tsilidis, Heather Ward, Elio Riboli, Elisabete Weiderpass, Michael F. Leitzmann.

Statistical expertise: Sebastian E. Baumeister, Sabrina Schlesinger, Krasimira Aleksandrova, Michael F. Leitzmann.

Administrative, technical, or logistical support: Carmen Jochem, Mazda Jenab, Pietro Ferrari, Marc J Gunter, Magdalena Stepien, Heinz Freisling, Elisabete Weiderpass, Tilman Kühn, Rudolf Kaaks, Bas Bueno de Mesquita, Giovanna Masala, Marie-Christine Boutron-Ruault, Franck Carbonnel, Agnès Fournier, Salvatore Panico, Roel Vermeulen, Anne May, María Dolores Chirlaque López, Francesca Fasanelli, Mireia Felez-Nobrega, Emily Sonestedt, Bodil Ohlsson, Elio Riboli.

## 1 ABSTRACT

2 **Background & Aims:** Evidence on the association between physical activity and risk of  
3 hepatobiliary cancers is inconclusive. We examined this association in the European Prospective  
4 Investigation into Cancer and Nutrition cohort (EPIC).

5 **Methods:** We identified 275 hepatocellular carcinoma (HCC) cases, 93 intrahepatic bile duct  
6 cancers (IHBC), and 164 non-gallbladder extrahepatic bile duct cancers (NGBC) among 467,336  
7 EPIC participants (median follow-up 14.9 years). We estimated cause-specific hazard ratios  
8 (HRs) for total physical activity and vigorous physical activity, performed mediation analysis, and  
9 secondary analyses to assess robustness to confounding (e.g., due to hepatitis virus infection).

10 **Results:** In the EPIC cohort, the multivariable-adjusted HR of HCC was 0.55 (95% confidence  
11 intervals (CI) 0.38-0.80) comparing active and inactive individuals. Regarding vigorous physical  
12 activity, for those reporting >2 hours/week compared to those with no vigorous activity, the HR  
13 for HCC was 0.50 (0.33-0.76). Estimates were similar in sensitivity analyses for confounding.  
14 Total and vigorous physical activity were unrelated to IHBC and NGBC. In mediation analysis,  
15 waist circumference explained about 40% and body mass index 30% of the overall association  
16 of total physical activity and HCC.

17 **Conclusions:** Findings suggest an inverse association between physical activity and risk of  
18 HCC, which is potentially mediated by obesity.

19 **Lay summary:** In a pan-European study of 467,336 men and women, we found that physical  
20 activity is associated with a reduced risk of developing liver cancers over the next decade. This  
21 risk was independent of other liver cancer risk factors, and did not vary by age, gender, smoking  
22 status, body weight, and alcohol consumption.

23

## 1 Graphical abstract

2 >

## 3 Highlights

- 4 • Liver cancer rates are increasing in Western countries, possibly due to increases in  
5 obesity, diabetes, and physical inactivity.
- 6 • Previous evidence was not convincing to support an effect of physical activity on liver  
7 cancer.
- 8 • We found that physical activity reduced the risk of hepatocellular carcinoma by about  
9 45%.

10

11 **Abbreviations:** BMI, body mass index; CI, confidence interval; DNA; deoxyribonucleic acid;  
12 EPIC, European Prospective Investigation into Cancer and Nutrition cohort; EPIC-PAQ,  
13 European Prospective Investigation into Cancer and Nutrition cohort physical activity  
14 questionnaire; g/d, grams per day; HBV, hepatitis B virus; HCV, hepatitis C virus; HCC,  
15 hepatocellular carcinoma; HR, hazard ratio; IHBC, intrahepatic bile duct cancers; MEDLINE,  
16 Medical Literature Analysis and Retrieval System Online; NGBC, non-gallbladder extrahepatic  
17 bile duct cancers; RR, relative risk; SD, standard deviation; US, United States of America; WCRF,  
18 World Cancer Research Fund International.

## 1    **Introduction**

2    Liver cancer was the fourth leading cause of cancer death in 2015 [1]. Liver cancer is responsible  
3    for around 47,000 deaths per year in the European Union [2]. Hepatocellular carcinoma (HCC)  
4    is the most common type of primary liver cancer derived from hepatocytes and it accounts for  
5    85-90% of all primary liver cancers worldwide. It is the fifth most common cancer in men and the  
6    seventh most common cancer in women [1]. The distribution of HCC varies greatly according to  
7    geographic location and it is more common in low- and middle-income countries than in  
8    developed countries. HCC more frequently occurs in Asia and Africa than in Europe and the US.  
9    The strongest risk factor for HCC is cirrhosis, a condition that is related to Hepatitis B virus (HBV),  
10    Hepatitis C virus (HCV), excessive consumption of alcohol, and exposure to aflatoxin B1 [1]. The  
11    geographic variability of HCC incidence has been widely associated to the different distribution  
12    of HBV and HCV infections [1, 3]. In high-income countries, the main risk factors for HCC are  
13    smoking, alcoholic cirrhosis, diabetes, obesity, and non-alcoholic hepatic steatosis [1, 4, 5]. The  
14    recent increase in HCC incidence is thought to be caused by increases in obesity, diabetes, and  
15    physical inactivity [6, 7]. The Physical Activity Collaboration of the National Cancer Institute's  
16    Cohort Consortium performed a pooled analysis of 10 prospective US and European cohorts and  
17    found that high compared to low leisure-time physical activity was associated with a 27% lower  
18    risk of liver cancer incidence [8]. Other prospective studies from the US and East Asian countries  
19    support an association of physical activity and lower risk of hepatobiliary cancers [8-13].  
20    However, the World Cancer Research Fund International judged that the evidence was not  
21    convincing to support an effect of physical activity on liver cancer [14]. Similarly, an umbrella  
22    review provided limited evidence for an association with liver cancer [15]. We report results from  
23    the EPIC (European Prospective Investigation into Cancer and Nutrition) cohort to provide  
24    additional evidence on the relationship between physical activity and HCC and other  
25    hepatobiliary cancers.

## 1   **Methods**

### 2   *Study Population and Data Collection*

3   The EPIC is a multinational prospective cohort study designed to investigate the link between  
4   diet, lifestyle and environmental factors with cancer risk and other chronic diseases. Detailed  
5   information on the study design, rationale, and methods of the EPIC cohort has been described  
6   previously [16]. Briefly, between 1992 and 2000, >520 thousand men and women, aged 25-70  
7   years, were recruited from 23 centers throughout 10 countries (Denmark, France, Germany,  
8   Greece, Italy, the Netherlands, Norway, Spain, Sweden, and the United Kingdom). Data on  
9   physical activity, education, smoking, alcohol consumption, coffee intake, anthropometric  
10   measurements and medical history were collected at baseline, before disease onset or diagnosis.  
11   All cohort members provided written informed consent. Ethics approval was obtained from the  
12   International Agency for Research on Cancer review board (Lyon, France) and participating  
13   centers. A total of 467,336 participants were included in the main analyses for total physical  
14   activity and hepatobiliary cancer risk after the following exclusions: 25,184 participants with  
15   prevalent cancer other than non-melanoma skin cancer; 20 subjects with missing date of  
16   diagnosis; and 4,128 individuals without follow-up. Four EPIC study centers (Naples, Umea,  
17   South-East of Norway, North-West of Norway) did not measure vigorous physical activity. Thus,  
18   the analysis of vigorous physical activity and hepatobiliary cancer risk was limited to 341,533  
19   participants for whom data on this exposure were available.

20   In a subset [17] of the EPIC cohort as of 2006, sera samples for HBV (ARCHITECT HBsAg,  
21   Abbott Diagnostics, France) and HCV (anti-HCV chemiluminescent microparticle immunoassays,  
22   Abbott Diagnostics, France) serologic tests were available: 115 HCC cases were matched using  
23   incidence density sampling to 230 controls based on age at blood collection, sex, study center,  
24   time of the day at blood collection, fasting status at blood collection; among women, additionally  
25   by menopausal status, and hormone replacement therapy use at time of blood collection. These  
26   data were used in nested case-control analyses to examine potential confounding by viral  
27   hepatitis status for the association of physical activity and HCC.



## 1 *Follow-up of Study Population and Case Ascertainment*

2 Incident first primary hepatobiliary cancer cases and vital status were ascertained through record  
 3 linkage with cancer and death registries in most centers [16]. In France, Germany and Greece,  
 4 ascertainment was done using a combination of methods including health insurance records,  
 5 pathology registries and active follow-up through mailed questionnaires/telephone interviews  
 6 [16]. Incident cancers were subsequently verified through medical records, pathology reports and  
 7 discharge diagnosis [16]. In all centers, cancer diagnosis required confirmation through  
 8 comprehensive pathology review [16]. A detailed protocol entitled 'Guidelines for Collection of  
 9 End-point Data in the EPIC study' for the collection and standardization of clinical and  
 10 pathological data for each cancer site was prepared by a special EPIC working group [16].  
 11 Cancer incidence was coded according to the International Classification of Diseases-Oncology-  
 12 2. HCC was defined as C22.0. Cancer of the intrahepatic bile duct (IHBC) was defined as C22.1.  
 13 Non-gallbladder extrahepatic bile duct tract cancer (NGBC) was defined as tumors in the  
 14 extrahepatic bile duct (C24.0), Ampulla of Vater (C24.1) or overlapping lesions of the biliary tract  
 15 (C24.8), and the biliary tract not specified (C24.9). We did not consider cancers of the gallbladder  
 16 (C23.9) as an endpoint because we assumed different underlying mechanisms [10].

## 17 *Assessment of Physical Activity*

18 The validated EPIC physical activity questionnaire (EPIC-PAQ) was used to assess recreational,  
 19 household and occupational physical activity during the past year in all EPIC centers, except in  
 20 the Norwegian centers [18-20]. Recreational physical activity was assessed by querying about  
 21 the amount of time in hours per week during the winter and summer spent with cycling and other  
 22 physical exercises (e.g., jogging, swimming) and was summarized into four groups: inactive,  
 23 moderately inactive, moderately active, and active [21, 22]. Participants reported their level of  
 24 occupational physical activity as either sedentary, standing, manual work or heavy manual work.  
 25 They were also asked whether engaging in household and recreational activities had caused  
 26 them to experience increases in sweating or heartbeat, and, if so, how many hours per week  
 27 they dedicated to these vigorous activities. We derived measures of total physical activity and  
 28 vigorous physical activity from the EPIC-PAQ. The Cambridge Index was used as a measure of

total physical activity by combining recreational physical activity and occupational physical activity [20, 22]. The Cambridge Index was developed [22] and validated [19] by comparing the EPIC-PAQ with objective measures of cardiorespiratory fitness and physical activity energy expenditure. The Spearman correlation between the Cambridge Index and physical activity energy expenditure was 0.33 (95% confidence interval: 0.28 to 0.38) [19]. The Norwegian EPIC centers measured total physical activity using a scale that ranged from 1 to 10 [23]; and the Cambridge Index for the Norwegian centers was derived as described previously [21]. Vigorous physical activity was categorized into 0,  $\leq 2$  (below the median), or  $> 2$  (above the median) hours per week [21, 24].

### *Statistical Analysis*

Hazard ratios (HR) and 95% confidence intervals (CI) were estimated using cause-specific Cox proportional hazard models, with age as the underlying time metric. Time of study entry was age at recruitment and exit time was age at cancer diagnosis or the last date at which follow-up was considered complete in each center. Models were stratified by center and sex to minimize departure from proportionality and to control for differences between centers, such as follow-up procedures and questionnaire design. Trend tests across exposure groups were performed by modeling the categorical physical activity variables as continuous covariables. We estimated cumulative incidence functions, adjusted for baseline confounders, accounting for competing risk of death from causes other than hepatobiliary cancer using a Fine-Gray subdistribution hazard model. The basic multivariable models were adjusted for education (no school degree, primary school, technical/professional/secondary, university), smoking status and intensity (never, current [1 to 15, 16 to 25, or  $\geq 26$  cigarettes/day], or former [ $\leq 10$  or  $> 10$  years]; current pipe, cigar or occasional smoking), current alcohol consumption (grams per day (g/d) modeled continuously using restricted cubic splines), lifetime alcohol use patterns (never, former,  $> 0 - 6$  [men]/ $> 0 - 3$  [women],  $> 6-12$  [men]/ $> 3-12$  [women],  $> 12-24$ ,  $> 24 - 60$ ,  $> 60$  g/d), and daily number of cups of coffee (1 cup was defined as 150 mL). For covariates with missing data (see Table 1), multiple imputation of covariates by fully conditional specification with accommodation of the substantive model [25] and 25 sets of imputed data was used. We examined multiplicative effect modification

1 by testing interaction terms of physical activity variables with sex, age (continuous), waist  
2 circumference (continuous), body mass index (continuous), baseline alcohol consumption  
3 (continuous) and lifetime alcohol consumption (categorical) using likelihood ratio tests; for  
4 continuous covariates a procedure based on fractional polynomials was used [26].

5 Because obesity and diabetes may be potential intermediates [4, 27, 28], our primary  
6 multivariable model did not control for them. Causal mediation analysis methods, as described  
7 for survival data [29], were used to examine the proportions of the association of physical activity  
8 with hepatobiliary cancer risk that was mediated by waist circumference, body mass index, and  
9 diabetes. These mediators were selected a priori based on subject knowledge [4, 27, 28] and  
10 were assessed using multiple linear regression (waist circumference, body mass index) and  
11 logistic regression (diabetes) for the mediator models and accelerated failure time models with  
12 Weibull distribution for time to event [29, 30]. Proportion mediated was calculated as indirect  
13 natural effect divided by the sum of the direct and indirect natural effect [29] and 500 simulations  
14 were used to derive quasi-Bayesian CI [30]. To facilitate the interpretation of mediation analyses,  
15 the categories 'active' vs. 'inactive' of the Cambridge Index and '>2 hours/week' vs. 'no' vigorous  
16 physical activity were compared. The mediation method assumes no unmeasured confounding  
17 in the exposure-outcome, mediator-outcome, and exposure-mediator relations, and no effect of  
18 the exposure on confounders of the mediator-outcome relation. We did not detect any exposure-  
19 mediator interactions.

20 We conducted several sensitivity analyses to test the robustness of our primary models. First, to  
21 minimize the influence of reverse causation, we excluded hepatobiliary cancer events that  
22 occurred during the first two years of follow-up. Second, although our primary analysis assumed  
23 that obesity and diabetes mediate the association of physical activity and hepatobiliary cancer  
24 risk, it is also plausible to hypothesize that overweight/obesity and diabetes render physical  
25 activity difficult (i.e., confound the association) [31]. Accordingly, we performed secondary  
26 analyses with additional adjustment for waist circumference and diabetes. Third, we assessed  
27 the robustness of observed associations to unmeasured confounding. Specially, we calculated  
28 E-Values [32], which indicate the minimum strength of association than an unmeasured

confounder would need to have with the exposure and the outcome on the risk ratio scale to fully account for an observed exposure-outcome association, above and beyond the measured covariates. Additionally, we used data from the EPIC nested case-control study [17] to adjust associations for HBV/HCV status. Odds ratios (OR) for HCC were derived from multivariable conditional logistic regression, adjusted for matching variables, age, sex, smoking status, current alcohol use, and coffee intake. Analysis of the nested case-control subset was performed among all subjects with additional adjustment for HBC/HCV; and among HBC/HCV negative individuals. Fourth, as an alternative to the stratified Cox model, we modeled unobserved heterogeneity across centers using a Cox model with a shared frailty. Fifth, due to different assessment of total physical activity in the Norwegian centers, we re-estimated our Cox models for total physical activity after excluding data from the Norwegian centers. Sixth, we performed complete cases analysis when covariates had missing values. P values < 0.05 are reported as statistically significant. Analyses were performed using R (version 3.5.1), SAS (version 9.4), and Stata (version 15.1).

## Results

### *EPIC Study*

#### *Characteristics of Participants*

Among the 467,336 participants in the EPIC study, the mean (SD) age was 51.3 (9.9) years, and 70.2% were women. During a median follow-up time of 14.9 years, participants contributed 6,508,182 person years, and 275 HCC, 93 IHBC, and 164 NGBC cancer cases occurred. Age-adjusted baseline characteristics of the analytical sample are provided in Table 1.

#### *Physical Activity and Hepatobiliary Cancer Risk*

Total physical activity and vigorous physical activity were inversely associated with HCC but not with IHBC and NBGC. The adjusted HR for HCC comparing 'active' and 'inactive' individuals was 0.55 (95% CI: 0.38 to 0.80, P for Trend < 0.001) (Table 2). The adjusted HR of HCC for '>2 hours/week' of vigorous activity vs. no vigorous activity was 0.50 (95% CI: 0.33 to 0.76, P for

Trend  $<0.001$ ) for HCC (Table 3). The adjusted cumulative incidence functions indicate that the physically inactive group showed excess HCC incidence compared to more active groups (Figure 1). The relations of total physical activity and vigorous physical activity with outcomes were not modified by sex, age, waist circumference, body mass index, smoking, current alcohol consumption or lifetime alcohol consumption (all  $P$  for interaction  $>0.1$ ).

#### *Mediation of the Association between Physical Activity and HCC Risk*

We used mediation analysis to estimate the proportions of the associations with HCC that were mediated by waist circumference, body mass index, and diabetes (Table 4). Waist circumference explained 40% and body mass index 30% of the overall association of total physical activity and HCC. The proportions of the total effect of vigorous physical activity on HCC mediated by waist circumference and body mass index were 17% and 12%, respectively. Diabetes did not seem to mediate the observed associations.

#### *Sensitivity Analyses*

In sensitivity analyses, the associations of total physical activity and vigorous physical activity with HCC, IHBC and NBGC were virtually unchanged when events occurring during the first two years of follow-up were excluded (Supplementary Tables 1 and 2). In models additionally adjusted for waist circumference and diabetes, the HR for HCC were attenuated but remained statistically significant. In the Cox model for total physical activity and HCC, for an unmeasured confounder to explain the HR estimate of 0.55, the unmeasured confounder would have to increase the likelihood of physical activity and decrease the likelihood of HCC by 3.0-fold, above and beyond the measured confounders. For an unmeasured confounder to bring up the upper confidence limit of 0.80 for this estimate to above 1.0, the unmeasured confounder would still have to both increase the likelihood of physical activity and decrease the likelihood of HCC by 1.8-fold, conditional on the measured covariates. Similarly, an unobserved confounder would need to be associated with a RR of 3.4 with vigorous physical activity and HCC to explain the estimated HR of 0.50 and a RR of 1.9 to move the upper confidence limit above 1.0, conditional on the measured covariates. We used the EPIC nested case-control study to perform additional

adjustment for HBV/HCV. The results of these analyses were similar in direction and magnitude to those reported for the entire cohort, but they were not statistically significant, due to small sample size (Supplementary Table 3). However, the data from the case-control dataset provide further support for the notion that additional confounding by HBV/HCV might not be sufficient to explain away the observed association of physical activity and HCC. Estimates from frailty models to account for between-center heterogeneity were similar those from the stratified Cox models. After exclusion of Norwegian centers and in complete case analyses, HR were almost identical to the primary analysis. The HR and CI from the complete case analyses were similar to those from primary models employing multiple imputation (Supplement Tables 1 and 2).

## Discussion

In this analysis of a multinational European cohort, higher total physical activity and vigorous physical activity were associated with lower risk of HCC. We observed a 45% lower risk of HCC when comparing high and low levels of total physical activity. The highest level of vigorous physical activity was associated with a 50% lower risk for HCC. Moreover, we observed that inverse associations of total physical activity and vigorous physical activity with HCC did not differ substantially between subgroups based on gender, lifestyle, and anthropometric variables. Findings from the sensitivities analyses suggest that the association of physical activity and HCC might be robust to reverse causation and unobserved confounding (e.g., by hepatitis virus infection). Our study also explored the roles of obesity and diabetes in physical activity's association with HCC. Our findings indicate that waist circumference mediated about 40% and BMI about 30% of the overall association of total physical activity and HCC. In contrast, diabetes did not seem to play an important role as a mediating factor.

These findings are in line with a pooled analysis of 10 cohorts with a total of 1,384 cases that reported a 27% lower risk of liver cancer comparing high and low levels of leisure time physical activity [8]. In the NIH-AARP Diet and Health Study, high versus no vigorous physical activity was related to a 44% lower risk of HCC [10]. Similar to our study, no association between physical activity and biliary tract cancer was shown in a previous analysis of NIH-AARP Diet and Health Study [10].

Several biological mechanisms might explain the inverse association between physical activity and hepatobiliary cancer, including systemic and local effects [28, 33]. The interrelated mechanisms most extensively studied are changes in whole-body and visceral fatness, metabolic dysregulation (e.g., insulin, glucose, insulin-like growth factors), adipokines (e.g., leptin, adiponectin), sex hormones (e.g., estrogen, testosterone), chronic low-grade inflammation, oxidative stress causing DNA damage and gene mutations (e.g., tumor suppression genes), impaired immune function, diluting effects on carcinogenic bile acids, and decreased intestinal transit time [33-35]. Evidence from prospective observational studies and randomized controlled trials suggests that the most relevant mechanism by which physical activity positively affects liver cancer risk is lowering body weight [27, 36-38]. The present study systematically explored the role of markers of overall adiposity (BMI), indirect measures of central obesity (waist circumference) and metabolic dysregulation (diabetes) in the overall association between physical activity and HCC. We found that central obesity might account for a large proportion of the direct effect of physical activity on HCC. The mechanisms underlying the association between central obesity and hepatobiliary cancer, particularly HCC, may occur through accumulation of excessive liver fat that increases pro-inflammatory molecules, leptin, and adiponectin [27].

The analysis of this large multinational European cohort provided sufficient events to examine the association of physical activity with hepatobiliary cancers. The cohort study also provided first insights into the relative importance of different intensities of physical activity. We performed sensitivity analyses to address potential selection bias, differences in case ascertainment between centers, and additional unobserved confounding. Although HBV and HCV are considered among the strongest risk factors for HCC [3], previous studies [8-13, 37] were unable to adjust for HBV and HCV. In the EPIC nested case control study the size and direction of the effect size for the association of physical activity and HCC was similar to that of the entire EPIC cohort; however, it was not statistically significant. Our sensitivity analyses for unobserved confounding using E-Values [32] further support the notion that any unmeasured confounding would need to be substantial to explain the inverse association of physical activity and HCC. The study had additional limitations. We were not able to adjust for other potentially important confounding factors (e.g., pleiotropic effects of statins) and to examine the role of intermediate

phenotypes (non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, cirrhosis). Further, compared to the general population, women were overrepresented in our sample, although men have higher risk of HCC [39]. Another limitation is that we were not able to examine in detail the type, intensity and amount of physical activity needed to reduce HCC risk. Physical activity and anthropometric measures were assessed only once at baseline. Repeated measurements of physical activity, anthropometric measures, and other potential biological intermediates over time would have strengthened our understanding of the underlying mechanisms. A recent analysis of the NIH-AARP Diet and Health Study [9] revealed that consistent participation in physical activity throughout the life course might be needed to reduce the risk of liver cancer incidence. We performed mediation analysis for indirect effects acting through general and central obesity, but we were unable to study trajectories of physical activity and body weight that could help to better separate the role of obesity as a confounder and mediator of the association of physical activity and risk of hepatobiliary cancer [8].

In conclusion, our analysis suggests that physical activity reduces risk of HCC. Studies with more detailed and objectively measured physical activity assessed at multiple time points throughout the life course are warranted to confirm our findings and may help establish the optimal dose, type, intensity, and timing of physical activity that is needed to prevent HCC.



## 1 **Acknowledgments:**

2 We thank all study participants and members of the EPIC study teams.

## 3 **Supplementary material**

4 Supplementary Tables can be found in the online version of the article.

## 5 **References**

- 6 [1] Akinyemiju T, Abera S, Ahmed M, Alam N, Alemayohu MA, Allen C, et al. The Burden of  
7 Primary Liver Cancer and Underlying Etiologies From 1990 to 2015 at the Global, Regional, and  
8 National Level: Results From the Global Burden of Disease Study 2015. *JAMA oncology*  
9 2017;3:1683-1691.
- 10 [2] Blachier M, Leleu H, Peck-Radosavljevic M, Valla DC, Roudot-Thoraval F. The burden of  
11 liver disease in Europe: a review of available epidemiological data. *Journal of hepatology*  
12 2013;58:593-608.
- 13 [3] Choo SP, Tan WL, Goh BKP, Tai WM, Zhu AX. Comparison of hepatocellular carcinoma  
14 in Eastern versus Western populations. *Cancer* 2016;122:3430-3446.
- 15 [4] Reeves HL, Zaki MY, Day CP. Hepatocellular Carcinoma in Obesity, Type 2 Diabetes, and  
16 NAFLD. *Digestive diseases and sciences* 2016;61:1234-1245.
- 17 [5] Saran U, Humar B, Kolly P, Dufour JF. Hepatocellular carcinoma and lifestyles. *Journal of*  
18 *hepatology* 2016;64:203-214.
- 19 [6] White DL, Thrift AP, Kanwal F, Davila J, El-Serag HB. Incidence of Hepatocellular  
20 Carcinoma in all 50 United States, From 2000 Through 2012. *Gastroenterology* 2016.
- 21 [7] Torre LA, Siegel RL, Ward EM, Jemal A. Global Cancer Incidence and Mortality Rates and  
22 Trends--An Update. *Cancer epidemiology, biomarkers & prevention : a publication of the*  
23 *American Association for Cancer Research, cosponsored by the American Society of Preventive*  
24 *Oncology* 2016;25:16-27.
- 25 [8] Moore SC, Lee IM, Weiderpass E, Campbell PT, Sampson JN, Kitahara CM, et al.  
26 Association of Leisure-Time Physical Activity With Risk of 26 Types of Cancer in 1.44 Million  
27 Adults. *JAMA internal medicine* 2016;176:816-825.
- 28 [9] Arem H, Lofffield E, Saint-Maurice PF, Freedman ND, Matthews CE. Physical activity  
29 across the lifespan and liver cancer incidence in the NIH-AARP Diet and Health Study cohort.  
30 *Cancer medicine* 2018;7:1450-1457.
- 31 [10] Behrens G, Matthews CE, Moore SC, Freedman ND, McGlynn KA, Everhart JE, et al. The  
32 association between frequency of vigorous physical activity and hepatobiliary cancers in the NIH-  
33 AARP Diet and Health Study. *European journal of epidemiology* 2013;28:55-66.
- 34 [11] Wen CP, Wai JP, Tsai MK, Yang YC, Cheng TY, Lee MC, et al. Minimum amount of  
35 physical activity for reduced mortality and extended life expectancy: a prospective cohort study.  
36 *Lancet (London, England)* 2011;378:1244-1253.
- 37 [12] Yun YH, Lim MK, Won YJ, Park SM, Chang YJ, Oh SW, et al. Dietary preference, physical  
38 activity, and cancer risk in men: national health insurance corporation study. *BMC cancer*  
39 2008;8:366.
- 40 [13] Inoue M, Yamamoto S, Kurahashi N, Iwasaki M, Sasazuki S, Tsugane S. Daily total  
41 physical activity level and total cancer risk in men and women: results from a large-scale  
42 population-based cohort study in Japan. *American journal of epidemiology* 2008;168:391-403.
- 43 [14] World Cancer Research Fund International, American Institute for Cancer Research. Diet,  
44 nutrition, physical activity and cancer: a global perspective. third expert report. Lyon; 2018.
- 45 [15] Rezende LFM, Sa TH, Markozannes G, Rey-Lopez JP, Lee IM, Tsilidis KK, et al. Physical  
46 activity and cancer: an umbrella review of the literature including 22 major anatomical sites and  
47 770 000 cancer cases. *British journal of sports medicine* 2018;52:826-833.

- [16] Riboli E, Hunt KJ, Slimani N, Ferrari P, Norat T, Fahey M, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. *Public health nutrition* 2002;5:1113-1124.
- [17] Trichopoulos D, Bamia C, Lagiou P, Fedirko V, Trepo E, Jenab M, et al. Hepatocellular carcinoma risk factors and disease burden in a European cohort: a nested case-control study. *Journal of the National Cancer Institute* 2011;103:1686-1695.
- [18] Haftenberger M, Schuit AJ, Tormo MJ, Boeing H, Wareham N, Bueno-de-Mesquita HB, et al. Physical activity of subjects aged 50-64 years involved in the European Prospective Investigation into Cancer and Nutrition (EPIC). *Public health nutrition* 2002;5:1163-1176.
- [19] Peters T, Brage S, Westgate K, Franks PW, Gradmark A, Tormo Diaz MJ, et al. Validity of a short questionnaire to assess physical activity in 10 European countries. *European journal of epidemiology* 2012;27:15-25.
- [20] Pols MA, Peeters PH, Ocke MC, Slimani N, Bueno-de-Mesquita HB, Collette HJ. Estimation of reproducibility and relative validity of the questions included in the EPIC Physical Activity Questionnaire. *International journal of epidemiology* 1997;26 Suppl 1:S181-189.
- [21] Benjaminsen Borch K, Friedenreich CM, Ferrari P, Casagrande C, Slimani N, Hemon B, et al. An update on how to treat physical activity data in the EPIC study. Lyon, France: the International Agency for Research on Cancer (IARC); 2015.
- [22] Wareham NJ, Jakes RW, Rennie KL, Schuit J, Mitchell J, Hennings S, et al. Validity and repeatability of a simple index derived from the short physical activity questionnaire used in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. *Public health nutrition* 2003;6:407-413.
- [23] Borch KB, Ekelund U, Brage S, Lund E. Criterion validity of a 10-category scale for ranking physical activity in Norwegian women. *The international journal of behavioral nutrition and physical activity* 2012;9:2.
- [24] Gallo V, Vanacore N, Bueno-de-Mesquita HB, Vermeulen R, Brayne C, Pearce N, et al. Physical activity and risk of Amyotrophic Lateral Sclerosis in a prospective cohort study. *European journal of epidemiology* 2016;31:255-266.
- [25] Bartlett JW, Seaman SR, White IR, Carpenter JR. Multiple imputation of covariates by fully conditional specification: Accommodating the substantive model. *Statistical methods in medical research* 2015;24:462-487.
- [26] Royston P, Sauerbrei W. Interaction of treatment with a continuous variable: simulation study of power for several methods of analysis. *Statistics in medicine* 2014;33:4695-4708.
- [27] Aleksandrova K, Stelmach-Mardas M, Schlesinger S. Obesity and Liver Cancer. Recent results in cancer research *Fortschritte der Krebsforschung Progres dans les recherches sur le cancer* 2016;208:177-198.
- [28] Greenlee H. Physical Activity and Digestive System Cancer Risk: Still Chasing the Promise. *JAMA oncology* 2016;2:1129-1131.
- [29] Valeri L, VanderWeele TJ. SAS macro for causal mediation analysis with survival data. *Epidemiology (Cambridge, Mass)* 2015;26:e23-24.
- [30] Tingley D, Yamamoto T, Hirose K, Keele L, Imai K. Mediation: R package for causal mediation analysis. *Journal of Statistical Software* 2014;59:1-38.
- [31] Wade KH, Richmond RC, Davey Smith G. Physical activity and longevity: how to move closer to causal inference. *British journal of sports medicine* 2018;52:890-891.
- [32] VanderWeele TJ, Ding P. Sensitivity Analysis in Observational Research: Introducing the E-Value. *Annals of internal medicine* 2017;167:268-274.
- [33] Ruiz-Casado A, Martin-Ruiz A, Perez LM, Provencio M, Fiuza-Luces C, Lucia A. Exercise and the Hallmarks of Cancer. *Trends in cancer* 2017;3:423-441.
- [34] Lucia A, Ramirez M. Muscling In on Cancer. *The New England journal of medicine* 2016;375:892-894.
- [35] Hojman P. Exercise protects from cancer through regulation of immune function and inflammation. *Biochemical Society transactions* 2017;15:905-911.
- [36] Berzigotti A, Saran U, Dufour JF. Physical activity and liver diseases. *Hepatology (Baltimore, Md)* 2016;63:1026-1040.
- [37] Keum N, Bao Y, Smith-Warner SA, Orav J, Wu K, Fuchs CS, et al. Association of Physical Activity by Type and Intensity With Digestive System Cancer Risk. *JAMA oncology* 2016;2:1146-1153.

- 1 [38] Giovannucci E. An Integrative Approach for Deciphering the Causal Associations of  
2 Physical Activity and Cancer Risk: The Role of Adiposity. Journal of the National Cancer Institute  
3 2018;110:935-941.
- 4 [39] Ozakyol A. Global Epidemiology of Hepatocellular Carcinoma (HCC Epidemiology).  
5 Journal of gastrointestinal cancer 2017;48:238-240.
- 6

Table 1 Age-adjusted Baseline Characteristics of the EPIC Cohort by Total Physical Activity (n = 467,336)

		Total physical activity (Cambridge Physical Activity Index)			
	<i>Total N</i>	Inactive	Moderately inactive	Moderately active	Active
Vigorous Physical Activity (%)					
None	182,178	55.9	42.5	30.0	28.5
≤2 hours/week	88,245	18.1	19.9	19.0	18.7
>2 hours/week	71,110	11.2	14.6	17.0	17.1
Missing	125,803	14.8	23.0	34.0	35.7
Sex (%)					
Men	139,168	26.8	27.7	27.5	40.4
Women	328,168	73.2	72.3	72.5	59.6
Education (%)					
No school degree/ unknown	20,859	7.3	3.7	3.6	4.2
Primary school	120,284	35.7	23.1	23.1	25.8
Technical/professional/secondary	198,720	40.0	43.7	43.8	43.6
University	112,121	15.6	26.3	26.8	24.1
Missing	10,658	1.3	2.6	2.6	2.3
Smoking (%)					
Never	20,2567	48.6	43.5	40.9	40.5
Current					
<15 cigarettes/day	53,680	10.2	11.1	12.1	12.9
≥15 cigarettes/day	37,534	9.4	7.7	7.4	7.9
Current pipe, cigar or occasional smoking	40,040	7.3	9.6	9.4	6.8
Former					
<10 years	44,584	8.2	9.4	9.9	10.8
≥10 years	75,403	13.6	16.0	16.9	18.4
Missing	13,528	2.6	2.7	3.4	2.8

Baseline alcohol consumption (g/d)		3.1	5.8	5.4	7.3
Average Lifetime alcohol consumption (g/d)					
Non-drinkers	28,146	8.7	6.4	4.3	4.6
Former	17,026	5.1	3.9	2.7	2.9
>0 – 6 (M)/> 0 – 3 (W)	93,442	25.4	21.3	16.3	17.2
>6-12(M)/>3-12(W)	110,070	24.6	24.3	22.2	22.6
>12-24	63,487	12.0	13.4	14.3	14.2
>24 – 60	41,822	7.2	8.6	10.1	9.8
>60	8,977	1.5	1.8	2.2	2.2
Missing	104,366	15.4	20.2	27.8	26.4
Coffee (ml/d)		179.3	281.1	316.9	409.4
Waist circumference (cm)		87.2	83.3	82.9	84.2
Missing	108,439				
Body mass index (kg/m <sup>2</sup> )					
Missing	82,692	26.4	25.1	24.8	24.9
Diabetes (%)		5.4	2.6	2.0	1.9
Missing	36,517				

EPIC, European Prospective Investigation into Cancer and Nutrition. Entries are adjusted medians for continuous variables and adjusted percentages for categorical variables. Adjustment for age using median regression (continuous covariates), binary logistic regression (dichotomous covariates), ordinal logistic regression (ordered categorical covariates), multinomial logistic regression (unordered categorical covariates)

Table 2 Association of Total Physical Activity and Hepatocellular Carcinoma (HCC), Intrahepatic Bile Duct Cancers (IHBC) and Non-Gallbladder Biliary Tract Cancer (NGBC) Risk in the EPIC cohort (n = 467,336)

	Total Physical Activity (Cambridge Index)				<i>P</i> Value for Trend
	Inactive (Reference)	Moderately inactive	Moderately active	Active	
<b>HCC (n)</b>	91	83	48	53	
HR (95% CI)	1.00	0.65 (0.48-0.89)	0.49 (0.34-0.71)	0.55 (0.38-0.80)	<0.001
<b>IHBC (n)</b>	26	27	21	19	
HR (95% CI)	1.00	0.72 (0.41-1.26)	0.66 (0.36-1.21)	0.82 (0.43-1.53)	0.477
<b>NGBC (n)</b>	39	46	36	43	
HR (95% CI)	1.00	0.67 (0.43-1.05)	0.67 (0.42-1.08)	0.88 (0.55-1.39)	0.761

EPIC, European Prospective Investigation into Cancer and Nutrition. HCC, hepatocellular carcinoma (C22.0). IHBC, intrahepatic bile duct cancers (C22.1). Non-gallbladder extrahepatic bile duct tract cancer (NGBC, C24.0, C24.1, C24.8, C24.9). HR (cause-specific hazard ratio) from center-and sex stratified Cox proportional hazards model, age as time metric, adjusted for education, smoking, baseline alcohol consumption, lifetime alcohol consumption, coffee. Missing covariate data was imputed using multiple imputation.

Table 3 Association of Vigorous Physical Activity and Hepatocellular Carcinoma (HCC), Intrahepatic Bile Duct Cancers (IHBC) and Non-Gallbladder Biliary Tract Cancer (NGBC) Risk in the EPIC cohort (n = 341,533)

HR (95% CI)	Vigorous Physical Activity			<i>P Value for Trend</i>
	None (Reference)	≤2 hours/week	>2 hours/week	
<b>HCC (n)</b>	122	33	32	
HR (95% CI)	1.00	0.50 (0.33-0.75)	0.50 (0.33-0.76)	<0.001
<b>IHBC (n)</b>	46	11	14	
HR (95% CI)	1.00	0.52 (0.26-1.06)	0.75 (0.39-1.44)	0.271
<b>NGBC (n)</b>	64	26	24	
HR (95% CI)	1.00	0.78 (0.47- 1.30)	0.80 (0.48-1.35)	0.368

EPIC, European Prospective Investigation into Cancer and Nutrition. HCC, hepatocellular carcinoma (C22.0). IHBC, intrahepatic bile duct cancers (C22.1). Non-gallbladder extrahepatic bile duct tract cancer (NGBC, C24.0, C24.1, C24.8, C24.9). HR (cause-specific hazard ratio) from center-and sex stratified Cox proportional hazards model, age as time metric, adjusted for education, smoking, baseline alcohol consumption, lifetime alcohol consumption, coffee. Missing covariate data was imputed using multiple imputation.

Table 4 Mediation Analysis for the Association of Total Physical Activity and Vigorous Physical Activity and Hepatocellular Carcinoma (HCC) in the EPIC cohort

Mediator	Total Physical Activity (Cambridge Index) (n = 363,228)		Vigorous Physical Activity (n = 275,433)	
	Proportion Mediated, %	P Value for Indirect Effect	Proportion Mediated, %	P Value for Indirect Effect
Waist Circumference	40.0	0.02	16.7	0.01
Body Mass Index	29.7	0.02	11.9	<0.01
Diabetes	4.2	0.21	0.6	0.23

EPIC, European Prospective Investigation into Cancer and Nutrition. HCC, hepatocellular carcinoma (C22.0). Adjusted for age, sex, education, smoking, baseline alcohol consumption, lifetime alcohol consumption, and coffee intake. Complete-case analysis was used for mediation analysis.



Figure 1 Cumulative Incidence of HCC according to Total Physical Activity and Vigorous Physical Activity

Adjusted cumulative incidence from a Fine-Gray model, with age as time metric, adjusted for education, smoking, baseline alcohol consumption, lifetime alcohol consumption, and coffee.